binds an epitope of BoNT/A, allowing the antibody to bind to BoNT/A to form an immunological complex, and detecting the formation of the immunological complex and correlating presence or absence of the immunological complex with presence or absence of BoNT/A in the sample. The sample can be biological, environmental, or a food sample.

[0008] Yet another aspect of the present invention is a kit for detecting BoNT/A in a sample. The kit includes a container holding an antibody which binds to an epitope of BoNT/A and instructions for using the antibody for the purpose of binding to BoNT/A to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of BoNT/A.

[0009] In still another aspect of the invention is provided a method for identifying the principal protective antigenic determinant of an antigen. The method includes making neutralizing antibodies using the complete antigen, reacting the neutralizing antibodies to different overlapping fragments encompassing the complete antigen, identifying a fragment of the antigen to which most neutralizing antibodies bind, narrowing the region containing the determinant by reacting the neutralizing antibodies to smaller regions of the identified fragment, and identifying the regions to which the neutralizing antibodies bind as the principal protective antigenic determinant.

[0010] In yet another aspect of the invention is provided BoNT/A-Hc peptides which have been found to be part of the principal protective antigenic determinants identified as SEQ ID NO:1 and SEQ ID NO:2. The peptides can be used singly or in combination, or the peptide sequences can be combined to produce one sequence and used as one peptide. These peptides may be useful as immunogens, and as a vaccine for protecting against BoNT/A intoxication.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

[0012] **FIG. 1**. Neutralizing MAbs recognize denatured BoNT/A Hc. BoNT/A Hc (1 mg/lane) was run on denaturing reducing gel and electroblotted onto a nitrocellulose membrane. The blotted paper was cut into 12 pieces and each segment was incubated with each MAb. The bound antibody was detected using color substrate. Immunopurified rabbit anti-BoNT/A Hc and SEB-2Ag were positive and negative control, respectively. C-fragment denotes BoNT/A Hc.

[0013] **FIG. 2**. The MAbs immunopercipitate BoNT/A Hc. BoNT/A Hc (1 mg/lane) was incubated with anti-BoNT/A Hc MAbs or anti-SEB (SEB-2Ag) MAb and the MAbs were immunoprecipitated on protein A-Sepharose. The proteins were separated on denaturing reducing gel and electroblotted onto a nitrocellulose membrane. The proteins were blotted with immunopurified rabbit polyclonal anti-BoNT/A Hc and the bound antibody was visualized using a Bio-Rad peroxidase development kit. C-fragment denotes BoNT/A Hc.

[0014] **FIG. 3**. Affinity mesurement of the neutralizing MAbs. Two representative of the binding analysis depicted here. Each MAbs was immobilized onto biosensor chip, after a wash-out phase, different concentrations of BoNT/A Hc were used to measure K-on and k-off.

[0015] **FIG. 4**. The MAb 6B2-2 binds to a distinct epitope on BoNT/A. Epitope mapping of the MAbs was carried out by SPR, Affinity-purified antibody to mouse IgG Fc was immobilized onto the chip. Purified-BoNT/A HC MAb was captured by the antibody, and then nonspecific sites were blocked by passing a saturating concentration of an unrelated antibody over the matrix surface. BoNT/A HC (200 nM) in HEPES buffered saline was passed over the antibodies at a flow rate of 5 μl/min. Finally, the second (competing) MAb was injected, and its binding determined. The biosensor chip was regenerated and the process was repeated to test the ability of all MAbs to bind as second MAb using each MAb as first MoAb.

[0016] **FIG. 5**. Neutralizing MAbs recognize two distinct binding sites and one overlapping epitope. MAbs 4A2-2, 4A2-4, 6E9-1, 6E9-3, 6E9-4, 6E10-4, 6E10-5, 6E10-8, and 6E10-10 recognize the same epitope. These antibodies recognize an overlapping epitope as 6C2-4. The MAb 6B2-2 recognizes a distinct epitope.

[0017] **FIG. 6**. Neutralizing MAbs recognize the amino acid residues within the carboxyl-terminal end of the BoNT/A Hc. The proteins were incubated with anti-BoNT/A Hc MAbs (6E9-12, 4A2-2 or 6C2-2) or anti-SEB (SEB-2Ag) MAb and the MAbs were immunoprecipitated on protein A-Sepharose. The proteins were separated on denaturing reducing gel and electroblotted onto a nitrocellulose membrane. The proteins were blotted with rabbit anti-BoNT/A Hc, peptide corresponding to N-terminal portion of BoNT/A Hc amino acid residues 915-1059 and peptide corresponding to amino acid residues within the carboxyl-terminal end of the BoNT/A Hc residues 1150-1289.

[0018] **FIG. 7**. Location of amino-acid residues corresponding to the designed peptides. The figure represents the X-ray crystallographic structure of TeNT-HC on which the positions of amino acids corresponding to peptide 1 and peptide 2 are distinguished. Panel (A) shows the alpha-carbon backbone tracing or, panel (B) depicts the molecular surface of TeNT-HC.

[0019] **FIG. 8**. Binding of protective MAbs to designed peptides. The ability of MAbs to recognize peptide 1 (A), peptide 2 (B) or control peptide (C) are depicted. The biotinylated-peptides were incubated with MAbs and then transferred to anti-IgG Fc coated ELISA wells. The bound biotinylated-peptide in complex with MAb was depicted using streptavidin.

[0020] **FIG. 9**. Immunogenicity of the 25-mer designed peptides. Groups of ten Balb/C mice were immunized with peptide 1, peptide 2, BoNT/A Hc or control peptide by i.p. injection (40 mg per mouse for peptides and 5 mg per mouse for BoNT/A Hc). The mice were boosted with the same immunizing dose at 3, 6 and 9 weeks. Two weeks after the last immunization mice were bled and serum titres against BoNT/A Hc determined. Each filled oval represents an endpoint titer of a single animal. Data are presented as reciprocal serum dilutions resulting in the absorbance reading twice above negative control (ELISA wells contained either no BoNT/A Hc or no primary antibody). The figure shows antibody responses against peptide 1 (A), peptide 2 (B), BoNT/A Hc (C) or control peptide (D).